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The scent glands of certain PHYCITIDAE (Lepidoptera). By Miss G. R. DICKINS, B.Sc., D.I.C. . . . . pp. 331–362

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THE SCENT GLANDS OF CERTAIN PHYCITIDAE (LEPIDOPTERA)

By Miss G. R. Dickins, B.Sc., D.I.C.

[Read 4th March, 1936.]

With Twenty-seven Text-figures.

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1. Introduction.

The fact that moths and butterflies emit an odour for the attraction of the opposite sex has been known for many years. Early workers and amateur collectors used this knowledge to good advantage and there are many instances on record where an unfertilised female has been placed in a gauze-covered cage in an open window, in order to attract males of the same species (Seitz,

1894; Urbahn, 1913). It is noteworthy that with few known exceptions the female always attracts the male; the reason is obvious when one considers the wingless females of the PSYCHIDAE and the heavy inert females of the BOMBYCIDAE, etc., which could not readily seek the males. The exceptions include *Hepialus hectus*, *H. humuli* (Degeener, 1902), possibly *Biston hirtarius* (Richards) and *Euploea core asela* (Latter, 1935).

Towards the latter half of the nineteenth century a few attempts were made to associate the scents of some of the well-known and conspicuous moths and butterflies with abdominal hair tufts and androconia on the wings (Thomas, 1893; Müller, 1877, etc.; Bertkau, 1882 and 1888; Dalla-Torre, 1885; Dewitz, 1884–5; Haase, 1882). But apart from the treatises of Illig (1902), Urbahn (1913), Freiling (1909) and Stobbe (1912) and the recent histological investigations of Eltringham (1913 onwards) very little is known about the function of the scent apparatus.

The species of PHYCITIDAE (*Ephestia kühniella*, *E. cautella*, *E. elutella*, and *Plodia interpunctella*) used for this work are all small and inconspicuous and cannot be recognised by a characteristic odour perceptible to the human sense.

2. MATERIAL.

The four species of moth used for this work belong to the PHYCITIDAE. Since the middle of last century they have been well known as pests of stored products and they are reared very easily under laboratory conditions. All the specimens used were obtained from the Field Station of the Imperial College of Science and Technology, where cultures of *Ephestia kühniella* on wholemeal flour, *E. cautella* and *E. elutella* on flaked maize and *Plodia interpunctella* on raisins, are maintained at standard conditions in the insectaries. Hence I have been fortunate in having a plentiful supply of fresh material always at hand. This made preservatives, which might have hindered fixation and the subsequent treatment prior to serial section cutting, unnecessary.

3. METHODS AND TECHNIQUE.

In examination of the position and morphology of the scent scales, the four distal segments of the abdomen in both sexes were dissected off, boiled in caustic potash, stained in carbol-fuschin and mounted whole. In this way the relative position of the modified scale tufts to the genitalia could be ascertained in the male, while the glandular nature of the intersegmental membranes of the ovipositor became apparent in the female.

The histological detail of the scales and the glands was examined by serial sections. In the beginning difficulty was experienced in sectioning the terminal segments owing to the chitinous genitalia of the male, and the internal struts of the ovipositor in the female. Different strengths of Eau de Javelle, caustic potash (cold and hot), and Eau de Labarraque for varying lengths of time were tried without success as chitin softeners. Similarly specimens were left soaking in Diaphanol for periods ranging from 2 to 50 hours, but in every case the histological detail was marred by the application of the reagent. It was finally concluded that the only way of overcoming the difficulty of chitin was to avoid hardening agents, and to use a method of double embedding with long infiltration. Boycott's celloidin-in-clove-oil method has proved quite satisfactory and has the advantage of being comparatively rapid.

Bouin (cold and hot), modified Bouin and Urea, and Carnoy, were tried as fixatives. It was found that material showed the best fixation when left for

24 hours in Carnoy, which besides penetrating more rapidly and more evenly than Bouin, has the additional advantage of containing no formalin.

Sections were cut in 56 m.p. wax at a thickness of 3–7 μ for transverse sections of adults, and at 5–13 μ for longitudinal sections of adults and for transverse sections of pupae.

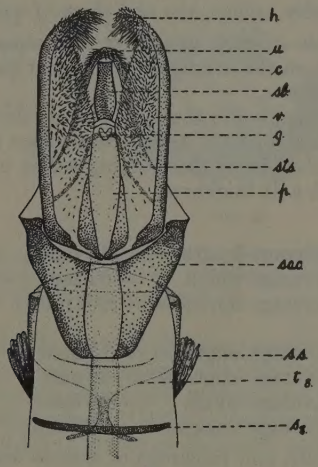


FIG. 1

FIG. 2.

FIGS. 1–2*b*.—(1) A ventral view of the terminal abdominal segments of *Ephestia kühniella* Z., ♂. *c.* costa; *g.* gnathos; *h.* harpes; *p.* penis; S_8. eighth sternite; *sac.* saccus; *sb.* subscaphium; *ss.* scent scales; *sts.* struts; t_8. eighth tergite; *u.* uncus; *v.* vale. (2*a*) A transverse section of a scent scale of *E. kühniella*, ♂. *l.* lumen; *p.s.* parallel striae; *s.m.* spongy matrix. (2*b*) A transverse section of a clothing scale taken from the eighth abdominal segment of *E. kühniella*, ♂.

Delafield's haematoxylin counterstained with eosin in 90% alcohol, and borax-carmine counterstained with picro-nigrosin, proved to be the best stains.

4. Description of Scent Apparatus.

Ephestia kühniella. Male. Position of scales.

The scent apparatus in the male of *E. kühniella* consists of two large and well-defined scale tufts present dorso-laterally on the eighth abdominal segment. These broad fans of modified scales arise from small chitinous areas situated one on either side of the narrow anterior arm of the eighth tergite (fig. 1). Normally the brushes are hidden by the invagination of the eighth within the seventh segment. If the abdomen of a male is pinned, dorsal side uppermost on to dissecting wax, and the eighth segment gently pulled out from

its invaginated position, the two tufts of scent scales can be easily seen. Occasionally under such conditions it will be observed that each brush appears to consist of two distinct tufts. This is due to the fact that about a dozen of the scales on either side are slightly more dorsal in position than the bulk of the brush, but unlike *E. cautella* and *E. elutella* there is actually but one brush on either side.

Number, colour, size and shape of scales.

The number of scales in each pencil or tuft seems slightly variable for different individuals, but on the average there appear to be about 65, including the 11 or 12 mentioned above.

The scales are not pigmented in the same way as the clothing scales but are of a pale uniformly golden colour. Apart from colour and situation the scent scales may be distinguished from the clothing scales by their different shape and much greater length and thickness.

Scent scale :—

Average length = ·38 mm.
Average width = ·04 mm.
Average thickness = ·005 mm.

Clothing scale :—

Average length (*c*) = ·13 mm.
Average length (*b*) = ·32 mm.
Average width = ·06 mm.
Average thickness = ·002 mm.

(N.B. Measure of width and thickness of a scale was taken at its broadest point near the apex.)

The clothing scales are of two main types according to their differences in size, but the largest of them does not exceed the length of a scent scale (fig. 4*b* and 4*c*).

In transverse section a comparative idea of the relative thickness of a scent and clothing scale may be obtained (fig. 2*a* and 2*b*). The matrix of the former appears more solid and the lumen better defined, but this may be due to the fact that it is nearly three times thicker than the ordinary scale.

In shape a scent scale has a blunt apex, never cleft or partite as a clothing scale may be, and tapers more gradually to the base than the latter. In common with the clothing scales each scent scale arises from a small alveolus or socket, which on staining with carbol-fuchsin becomes very conspicuous on account of its densely chitinous nature.

Microscopic structure.

A section of a scale confirms the presence of longitudinal striation which may be observed by superficial examination. By the latter means, however, the striation of all scales appears very similar (fig. 4), but in reality there are some striking differences to be noted. In the first place, a scent scale is furnished on both faces with a series of parallel ridges which pass from the blunt apex to the point where the lamina joins the hollow pedicel. A clothing scale has the striation on one side only—the external face; the inner surface (that approximate to the body) being entirely smooth (fig. 2*b*). Secondly the ridges of the scent scale are of an even regular nature, whereas those on the clothing scale show irregularities in height and varying spaces or furrows between each ridge.

In examination of the finer histological details of the scales and the tissue

from which they originate, sections were made of both adults and pupae. Fig. 5 is a section of a male pupa showing the scent scales arising from two glandular areas on either side of the intersegmental space between the seventh and eighth abdominal segments. Under high microscopic power each scale is seen to originate in a large multinucleate gland cell with deeply staining

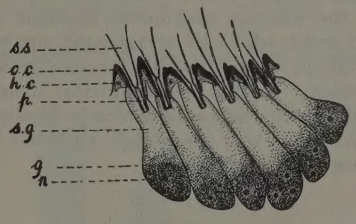


FIG 3.

FIG. 3.—An enlarged view of some of the scent glands from *E. kühniella*, ♂. *c.c.* chitinous cuticle; *g.* darkly staining granules; *h.c.* hypodermal cell; *n.* nucleus; *p.* petiole of scent scale; *s.g.* scent gland; *s.s.* scent scale.

granules at its base (fig. 3). Although these granules are much smaller and less numerous near the pore-like opening at the end of the scent scale stalk I can discover no well-defined vacuole as described by Illig (1902) in *Colias edusa* (male), and by Eltringham (1926*b*) in *Eryphanis polyxena*, nor secreting reservoir depicted by Freiling (1909) in *Stilpnotia salicis* (female).

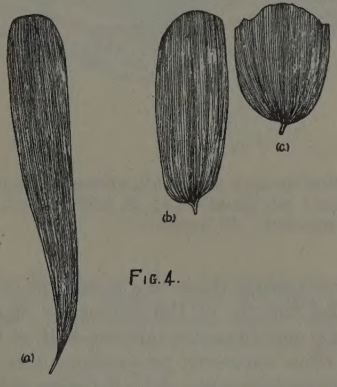


FIG. 4.

FIG. 4.—Some scales from *E. kühniella*, ♂. (*a*) scent scale; (*b*) and (*c*) clothing scales.

Between each scale alveolus and underlying the thick chitinous cuticle is the remains of a small hypoderm cell with finely granular protoplasm.

Female.

The scent organs of the female are extraordinarily simple in view of the fact that they are responsible for the enticing odour which is necessary for the continuance of the species.

It was noticed that when a male and female were placed in a closed box together, that the male became aware of the presence of the female only after she had adopted the "calling" attitude (Norris, 1932). This suggested the likelihood of there being a scent apparatus in the intersegmental membranes between the apical abdominal segments, similar in position to those described by Freiling and Urbahn in certain butterflies. When the ovipositor is completely retracted, the seventh abdominal segment encloses the terminal segments ten, nine and eight, which lie invaginated one within the other. But when the female is calling, the ovipositor frequently undergoes a rhythmic protrusion and retraction. If the intersegmental membranes had a scent-secreting hypodermis the extrusion would facilitate dissemination of the volatile substance; while if the scent was completely confined to scales, it might be liberated by the friction of the movement.

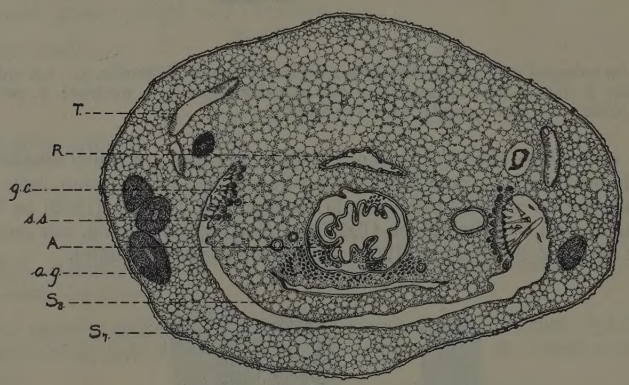


FIG. 5.

FIG. 5.—A transverse section through the eighth abdominal segment of a male pupa of *E. kühniella*. *A.* aedeagus; *g.c.* gland cells; *R.* rectum; *s.s.* scent scales; S_7. seventh segment; S_8. eighth segment; *T.* trachea.

With a view to investigating these two possibilities, the terminal segments were fully extended and the tip of the ovipositor fastened by a fine pin to dissecting wax. On the membranous anterior half of the eighth segment are numerous small, pale yellow anteriorly projecting scales. They are half hidden by the long pigmented partite scales which fringe the posterior edge of the seventh segment. Although they are scattered right round the base of the eighth segment, these small scales are particularly plentiful ventrally, where they are aggregated about the opening of the bursa copulatrix. If the ductus bursae be partially opened near the ostium, it may be seen that a few of these scales are scattered in the terminal chamber of the ductus. These scales are only about a quarter of the size of the scent scales in the male.

Average length = ·09 mm.
Average width = ·018 mm.

Broad clothing scale :—

Average length = ·16 mm.
Average width = ·05 mm.

Long fringing scale of seventh segment :—

Average length = ·35 mm.
Average width = ·03 mm.

Fig. 6 shows the comparative shapes and sizes of the above three types of scales.

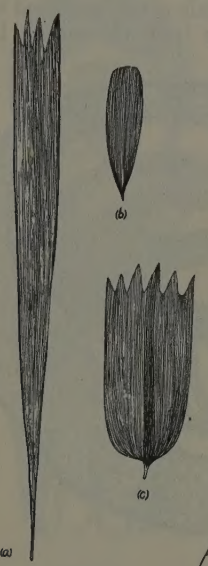


FIG. 6.

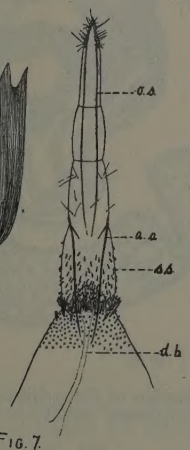


FIG. 7.

FIGS. 6–7.—(6) Scales from *E. kühniella*, ♀. (*a*) fringing scale from the seventh segment; (*b*) small scale; (*c*) ordinary clothing scale. (7) The extended ovipositor of *E. kühniella* showing the position of the scent scales. *a.a.* anterior apophyses; *d.b.* ductus bursae; *o.s.* ovipositor struts; *s.s.* small scales described in text.

Whether these scattered yellowish scales are scent producing is difficult to determine. From a careful study of serial sections it appears that underlying these scales there are no enlarged gland cells comparable with those found in the male. Examination of sections of female pupae throws no light on the problem, there being no precocious development and subsequent atrophy, prior to the emergence of the adult, of any recognised kind of gland cell.

Transverse sections show, however, that the intersegmental area between the eighth and ninth segments has just such a hypoderm as was sought for round the ostium bursae. Here the cells composing the hypoderm are large and well defined with conspicuous nuclei. The overlying cuticle is rugose

and papillated and much thicker than elsewhere. It has fine lamellated stratifications, but no pores or canals are visible (fig. 22). The most remarkable fact about this area is that ventrally it dips much deeper between the ninth and eighth segments than it does dorsally, so that sections anterior to its dorsal limit show the ventral extension as a closed sac (fig. 8). This condition is comparable with that found by Urbahn (1913) in *Phalera bucephala*, etc., and he considered it as the forerunner of the "glandular odoriferae" which are developed as invaginations of this intersegmentum (Jackson, 1890).

The assumption that this intersegmental membrane is scent producing is in accord with Eltringham's recent work on *Ocneria dispar*. He has found that the directive odour of the female is not produced by special glands as are the aphrodisiac odours emanating from the male. In place of a specially

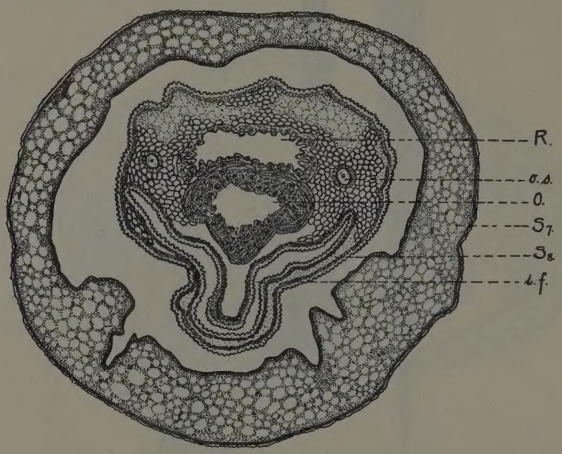


FIG 8.

FIG. 8.—A transverse section of the ovipositor of *E. kühniella*. *i.f.* intersegmental fold; *O.* oviduct; *o.s.* ovipositor struts; *R.* rectum; S_7. seventh segment; S_8. eighth segment.

differentiated gland is merely a thickened and modified hypoderm in which no actual pores are present. He therefore presumes that actual secretion of the volatile scent-producing substance takes place by a sort of diffusion or osmosis through the cuticle, a method which seems to be common in such structures, notably in the repulsive glands of the HELICONIIDAE.

Plodia interpunctella. Male. Position of scales.

Plodia interpunctella is closely allied to *Ephestia kühniella*, perhaps even more so than their different generic names would suggest. Hence it is not surprising to find the form and position of the scent apparatus to be very similar in both species. In *P. interpunctella* the two tufts of scent scales are inserted on a level with the cleft of the anterior arm of the eighth tergite. One frequently finds individual variation in the length and degree of chitinisation of the arm of the eighth tergite, so that in consequence the position of the modified scale

tufts is subject to slight alteration. Thus they may sometimes appear to be inserted above, and sometimes below, the anterior cleft. According to Richards and Thompson (1932), "on each side of the tergite anteriorly a tuft of long modified scales arises from a small chitinised area." But in all specimens examined I found the scales to be distributed in two tufts on either side, recalling the condition sometimes seen in *E. kühniella*. The two most conspicuous groups are composed of scales of diverse lengths, the longest reaching nearly to the anterior margin of the fan-shaped expansion of the eighth tergite. The other

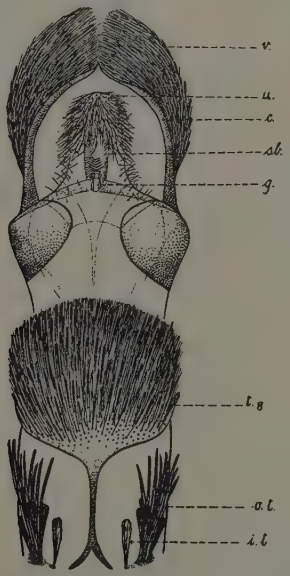


Fig. 9.

Fig. 9.—A dorsal view of the terminal abdominal segments of *Plodia interpunctella*, ♂. *c.* costa; *g.* gnathos; *i.t.* inner tuft of scent scales; *o.t.* outer tuft of scent scales; *sb.* subscaphium; t_8. eighth tergite; *u.* uncus; *v.* vale.

two tufts arise closer to the arm of the tergite and are small and easily overlooked (fig. 9).

Number, colour, size and shape of scales.

The number of scales in an outer tuft is on the average 50, while the inner appears to have invariably 9 or 10. Thus the total number of scales on either side is practically the same as in *E. kühniella*.

The colour of the scales in the large tufts varies from brown to almost black so that they stand in sharp relief against the white intersegmental membrane. In contrast the small inner scales are pale fawn or golden yellow. A transverse section clearly indicates this difference in colour, the largest tufts being composed of narrow black scales and wider golden brown scales (fig. 11).

The scales of the larger tuft are usually segregated into two distinct types.

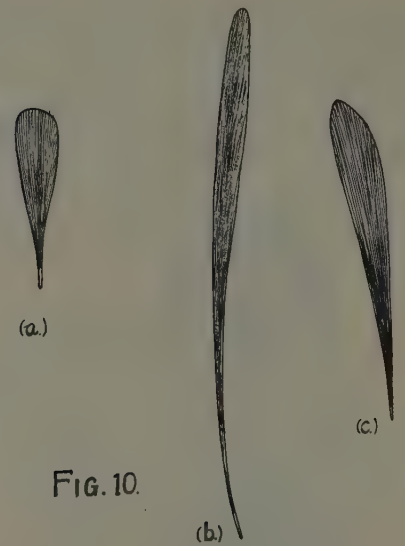


FIG. 10.—Scales from *P. interpunctella*, ♂. (*a*) scale from inner tuft; (*b*), (*c*) scales from outer tuft.

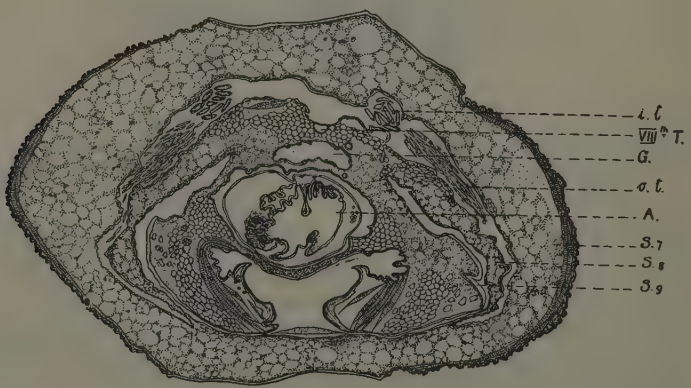


FIG. 11.—A transverse section through the invaginated terminal segments of *P. interpunctella*, ♂. *A.* aedeagus; *G.* gut; *i.t.* inner tuft of scent scales; *o.t.* outer tuft of scent scales; S_7, S_8, S_9. segments 7, 8 and 9; *VIII*th *T.* eighth tergite.

Apart from the colour difference mentioned above, they vary considerably in width, length and thickness (fig. 10). For convenience I shall designate the longest as scales " b " and " c," and the others as scales " a."

Scales of outer tuft :—

Black scales " b " :—

Average length	= ·54 mm.
Average width	= ·02 mm.
Average thickness	= ·003 mm.

Brown scales " c " :—

Average length	= ·33 mm.
Average width	= ·06 mm.
Average thickness	= ·002 mm.

Scales of inner tuft :—(*a*)

Average length	= ·21 mm.
Average width	= ·04 mm.
Average thickness	= ·004 mm.

In shape all scales of the scent apparatus have an undivided blunt apex, tapering to a narrow hollow pedicel.

Microscopic structure.

All the scales have regular striations on both faces. The pale scales of the small inner tufts have thick chitinous walls, with flattened striae, a dense peripheral matrix, and a well-defined lumen. Scales " b " and " c " have much thinner walls, very conspicuous widely spaced striae and their interiors are completely filled by a very fine chitinous reticulum.

As in *E. kühniella*, the intersegmental fold in which the scent pencils arise

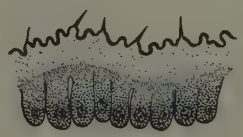


FIG. 12.

FIG. 12.—Gland cells from the intersegmental fold between the abdominal segments eight and nine of *P. interpunctella*, ♀.

is deeper dorsally than ventrally, hence in section resembling a closed pocket. The points of attachment of the small tufts are slightly more anterior to the large, but when the genitalia are retracted both appear deep within the fold.

Both tufts are subtended by deeply staining gland cells which underlie a thick chitinisation of the cuticle. The glands beneath the small inner groups are larger, the nuclei more prominent and the cytoplasm denser, than those from which scales " b " and " c " arise, but all show compression owing to the large development of muscle which surrounds them. The muscle fibres immediately below the glands are circular, but internal to these are numerous longitudinal fibres.

Female.

In describing the glandular fold in the female, little can be added to the description already given for *E. kühniella*. The hypoderm cells are columnar, heavily granulated at the base and with large nuclei containing two or three prominent nucleoli. Their inner cell walls are well defined but become less evident near the periphery, so that, unlike *E. kühniella*, the gland cells appear to merge into the chitinous cuticle. This layer of thick chitin has a spongy appearance and the fine laminae seen in *E. kühniella* are here scarcely discernible. The surface is beset with innumerable chitinous processes some of which are surmounted by fine spines (fig. 12).

Ephestia cautella. Male. Position of scales.

As in *E. kühniella* and *Plodia interpunctella* the scent scales in the male are dorsally situated on the eighth abdominal segment. When the apical segments are exserted, the scent scales can be seen as five modified tufts on each side of the tergite, with an additional dense median tuft arising from the membrane between the seventh and eighth tergites. In this extended condition the

FIG. 13.

FIG. 13.—Gland cells from the intersegmental fold between the abdominal segments eight and nine of *Ephestia elutella*, ♀.

median tuft and one of the tufts on either side project anteriorly, the rest being directed posteriorly (fig. 4). Of the latter, one on each side is very long and (even when the genitalia are retracted) the apices of its scales project slightly beyond the posterior edge of the seventh segment. In this position (*i.e.* genitalia retracted) the apices of all scales are directed posteriorly, so that they form an unbroken crescentic band of dark scales, plainly visible through the thin integument of the seventh tergite. (For convenience of description an arbitrary nomenclature will be adopted for each tuft (figs. 14 and 15).)

Number, colour, size and shape of scales.

With the exception of the dense median tuft of fine scales, and the somewhat similar scales of Group 1, the numbers in each tuft are comparatively easy to estimate accurately; moreover, the numbers appear to be subject to very little individual variation.

Numbers of scales in each tuft :—

Group 1 . . .	approx. 120.
Group 2 . .	25.
Group 3 . .	30.
Group 4 . . .	5.
Group 5 . . .	14.
Median Group .	approx. 500.

The colour of all tufts is definitely darker than any of the clothing scales, and externally they appear uniformly dark brown to black. In section, however, Group 4 are always seen to be decidedly paler and Group 3 usually darker than the rest. This difference in colour is due partly to the amount of pigmentation and partly to the degree of thickness of the scale walls. The

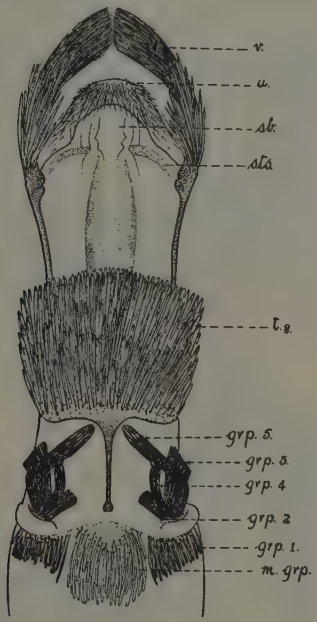


FIG. 14.

FIG. 14.—A dorsal view of the terminal abdominal segments of *E. cautella*, ♂. *grp.* 1, 2, 3, 4, 5, scent scales of groups 1, 2, 3, 4 and 5; *m.grp.* median group; *sb.* subscaphium; *sts.* struts; t_8. eighth tergite; *u.* uncus; *v.* vale.

distal ends of Group 5, which can just be seen externally, are paler than the hidden portion of the tuft, so that they harmonise with the ground-colour of the clothing scales.

Some idea of the relative proportions of the scales can be gleaned from fig. 15, and the following measurements indicate their actual sizes.

Average lengths :—

Group 1 . . .	·22 mm.
Group 2 . . .	·22 mm.
Group 3 . . .	·38 mm.
Group 4 . . .	·46 mm.
Group 5 . . .	·77 mm.
Median Group . .	·26 mm.

Average widths :—

Group 1 . . .	·054 mm.
Group 2 . . .	·045 mm.
Group 3 . . .	·06 mm.
Group 4 . . .	·035 mm.
Group 5 . . .	·04 mm.
Median Group . .	·02 mm.

Average thicknesses :—

Group 1 . . .	·003 mm.
Group 2 . . .	·008 mm.
Group 3 . . .	·004 mm.
Group 4 . . .	·008 mm.
Group 5 . . .	·007 mm.
Median Group . .	·001 mm.

Before a description of the shape of individual scales is given, mention must be made of the general appearance of each tuft. The Median Group and Group 1, being composed of numerous fine scales, give the effect of loose brushes.

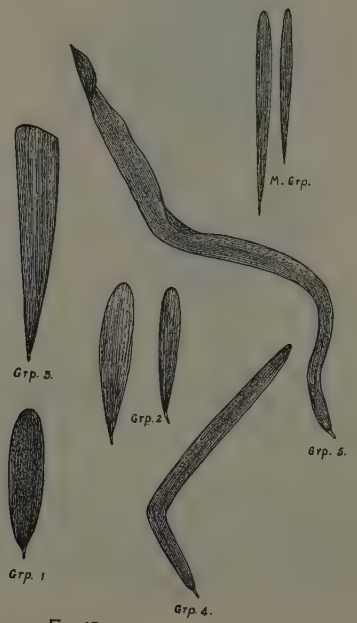


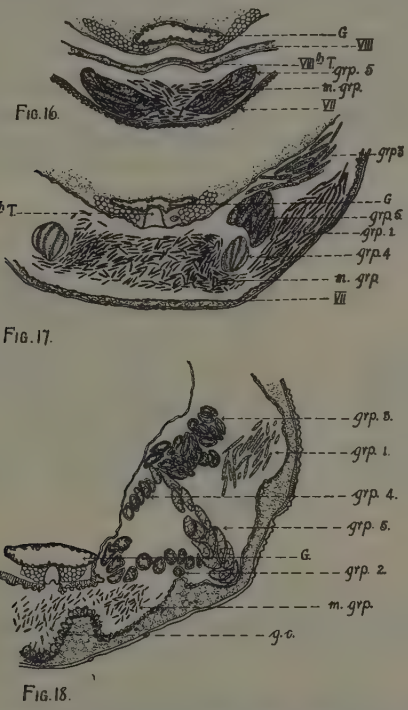
FIG. 15.

FIG. 15.—Scent scales from groups 1, 2, 3, 4 and 5, *E. cautella*, ♂.

Groups 2 and 3 have a rigid palisade appearance; while the component scales of Groups 4 and 5 cohere, giving the effect of two pencils. The scales of Group 5 have a definite interlocking device owing to their apices being folded over one another, and they are secured in a similar way at the elbow of the bend which

occurs about half-way down their length. The scales of Group 4 are bent into the form of a boomerang, but appear to lack a mechanical locking apparatus. I am of the opinion that they are held together by a sticky secretion.

Scales of Groups 1 and 2 have obtusely rounded apices and broad laminae which in the former do not taper gradually to the pedicel as in Group 2. Those of Group 3 have rectangular apices and contrastingly narrow bases, but the



FIGS. 16, 17, 18.—Transverse sections of the scent-scale tufts of *E. cautella*, ♂, taken at three different levels. Fig. 16 is the most posterior. *G.* gut; *g.c.* "goblet" cells; *grp.* 1, 2, 3, 4, 5, scent scales of groups 1, 2, 3, 4 and 5; *m.grp.* median group; *VII*, *VIII*, segments seven and eight; *VIII*th *T.* eighth tergite.

scales of Groups 4 and 5 maintain practically a uniform width from apex to base.

Microscopic structure.

Transverse sections of a modified scale show that as the width decreases, the thickness becomes correspondingly increased, so that near its point of insertion a scale appears almost circular in outline (fig. 18). Figs. 16, 17 and 18 are three transverse sections of an adult male *Ephestia cautella* cut at 9 μ. Fig. 16 is a section taken through the invaginated ninth segment and hence includes segments eight and seven into which it is telescoped. At this level only the scales of Group 5 and a few of the longest scales of the Median Group are apparent. The coherence of the scales of Group 5 and the fact that the

drawings are made with a $\frac{2}{3}$ objective, preclude the possibility of distinguishing the longitudinal striae, which under a $\frac{1}{12}$ objective are visible as minute serrations. Their interiors are filled with a chitinous reticulum which branches irregularly from the inner surfaces of the scale walls.

Fig. 17 is a section anterior to the ninth segment and just posterior to the apices of the scales of Group 2. In this figure all the groups with the exception of Group 2 can be distinguished, the similarity between the scales of Group 1 and the median tuft being quite apparent. The five tightly adpressed scales of Group 4 have at this level thin walls and a very fine primordial reticulum of chitin.

Fig. 18 is a section through the bases of most of the scales. Groups 2, 3 and 4 are cut just above the pedicels, so that they show thickened walls, a diminished network of chitin, and pronounced lumena which are continued into the cavities of the scale stalks. The pedicels of the scales of Groups 4 and

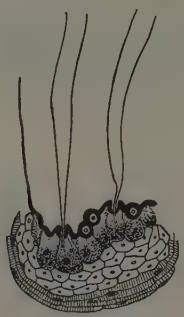


FIG. 19.

FIG. 19.—Part of a longitudinal section through the eighth abdominal segment of *E. cautella*, ♂, showing the gland cells subtending the scales of group 5.

5 are embedded in alveoli of extremely thick homogeneous chitin, immediately beneath which are the large deeply staining gland cells. The scales of Groups 1, 2 and 3 are subtended by very much smaller cells which have completely lost their columnar shape owing to the enormous development of circular muscle fibres which surround them. Both in transverse and longitudinal sections the larger size of the glands, from which Groups 4 and 5 arise, is evident. The scales of the median tuft are embedded in weakly chitinised sockets each of which communicates with a uninucleate cell. Each cell and its scale alveolus stand above the surface of the integument so that this whole area has a very rugose appearance, discernible even before sectioning or staining. These cells might be described as "goblet" in shape, but otherwise are in no way analogous to the goblet cells found scattered amongst columnar epithelium of vertebrate glands.

Female.

Just as the scent apparatus of the male of *E. cautella* has a far greater development than in *E. kühniella*, so does the female show a corresponding degree of complexity. As in the other species described the ventral fold

between segments eight and nine is lined by columnar, nucleated gland cells overlaid by a chitinous cuticle. The cells are much larger than in *E. kühniella* or *Plodia*, and they are frequently separated by interstices which occur near their bases. But although the individual cells may thus be clearly distinguished, the cell walls dividing their bases are completely obliterated, so that they appear to be merged into a thin common syncytium. This syncytial layer is only faintly granular and appears as a clear zone sharply demarking the gland cells from the chitinous stratum (fig. 20). Two or three tiny vacuoles may be present in close proximity to the large nucleus with its prominent nucleolus.

The development of the chitinous layer is ten times greater than in *kühniella*, and has the peculiar property of being able to absorb haematoxylin when the clothing scales and other chitinous structures remain completely unstained. Examination by an oil immersion objective shows that the usual homogeneous

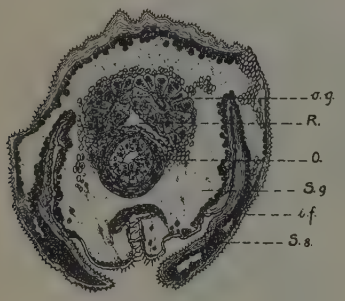


FIG. 21.

FIG. 22.

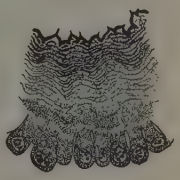


FIG. 20.

FIGS. 20–22.—(20) Gland cells from the intersegmental fold between the abdominal segments eight and nine of *E. cautella*, ♀. (21) A transverse section through the ninth abdominal segment of an advanced female pupa of *E. cautella*. *i.f.* intersegmental fold; *O.* oviduct; *o.g.* odoriferous gland; *R.* rectum; S_8, S_9. segments eight and nine. (22) Gland cells from the intersegmental fold between the abdominal segments eight and nine of *E. kühniella*, ♀.

character of chitin is here replaced by a spongy almost granular appearance. Lamellate stratifications are very evident and are particularly close together beneath the spined rugose epiderm. Scattered between the laminae small passages are noticeable which apparently correspond to the network of horizontal canals which Urbahn (1913) described in several female butterflies, and which Freiling (1909) presumes to be present in the intersegmental scent organs of *Orgyia antiqua* and *Bombyx mori*. There are no apparent vertical canals communicating with the exterior such as Illig (1902) demonstrated in certain SPHINGIDAE, and Wigglesworth (1934) depicts in *Rhodnius*, etc.

In a transverse section of a female *cautella*, either adult or advanced pupa (figs. 23 and 21), two large multicellular glands can be seen lying one on either side of the thin-walled rectum and dorsal to the common oviduct. They open into the oviduct almost on a level with the external genital pore, and extend inwards as two white freely hanging sacs the full length of the eighth segment. The walls of the sac are composed of a single layer of columnar cells which are faintly striated but show no obvious granules or vacuoles. They are distinctly

marked off from one another except near the lumen of the sac, where they are merged into a syncytium lying immediately beneath the chitinous layer. This lining of chitin is far thinner than that lining the intersegmental folds, yet it still shows the characteristic lamellate stratifications. Its surface is covered with shallow cup-shaped cavities which appear polygonal in outline. The ridges which separate these small depressions are seen in transverse section as acute irregular processes of the chitin. As these glandular sacs agree in every detail with what earlier workers have described as " glandulae odoriferae " I must include them in a description of the scent apparatus of *E. cautella*. Such odoriferous glands are not universally present in Lepidoptera, and although widely distributed among the various families, may occur sporadically in non-related genera. They were recorded for the first time by v. Siebold as early as 1837 in *Argynnis melitaea, Zygaena*, etc.; and Petersen (1900) found them in species of *Vanessa, Zygaena, Ortholitha* and *Eupithecia*. Jackson

Fig. 23.

Fig. 23.—A transverse section of the ovipositor of *E. cautella*, ♀. *i.f.* intersegmental fold; *O.* oviduct; *o.g.* odoriferous gland; *o.s.* ovipositor struts; *R.* rectum; S_7, S_8, S_9. segments seven, eight and nine.

(1890) gave a description of their development in *Vanessa io* :—" They arise in the pupa between five and six days old during the stage when the oviducal aperture of the azygos oviduct is shifting backwards towards the anus. The odoriferous glands develop as two tubular ingrowths of the hypodermis, one on the right, the other on the left of the middle line, perfectly distinct from one another; *i.e.* each of them has its own separate aperture to the exterior. The ingrowths are in front of, or anterior to the oviducal aperture. They are near the oviducal aperture and underlie the anterior margin of the vesicles of the sebaceous glands." Freiling apparently did not come across these glands, but Urbahn (1913) gave a full description of similar glandular structures in all four species of *Argynnis*. He stated that they are present in the last abdominal segment and open simultaneously with the vagina to the exterior. Moreover, he maintained that with an oil immersion lens large secretory vacuoles can be seen in the gland cells, but added that they are always very indistinct.

Ephestia elutella. Male. Position of scales.

On each side of the small anterior triangular expansion of the eighth tergite arise three tufts of modified scales, of which the inner is the shortest and

broadest, and the outer the longest. Richards and Thompson (1932) describe and figure a very small fourth tuft near the base of the intermediate group, but investigation has proved this to be the recurved bases of the middle tuft (fig. 24). In addition to these scent brushes are numerous very fine scales arising between the tufts and the seventh segment.

Number, colour, size and shape of scales.

The outer and inner tufts (Groups 1 and 3 respectively) have approximately the same number of scales, *i.e.* about 36; but the intermediate group (Group 2) is never composed of more than 10 scales, which are held tightly together, recalling the condition of Group 4 in the male of *E. cautella*. All the scales

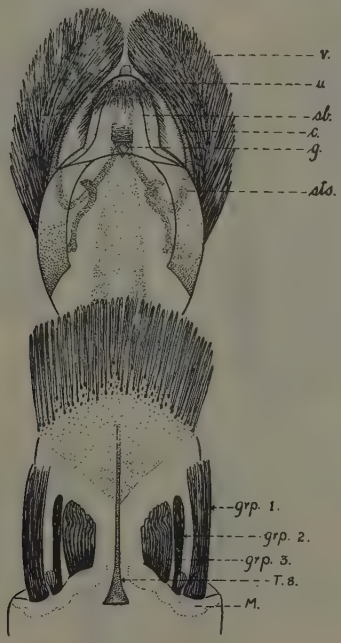


FIG. 24.

FIG. 24.—A dorsal view of the terminal abdominal segments of *E. elutella*, ♂. *c.* costa; *g.* gnathos; *grp.* 1, 2, 3, scent scale of groups 1, 2 and 3; *M.* muscle; *sb.* subscaphium; *sts.* struts; T_8. eighth tergite; *u.* uncus; *v.* vale.

are brown in colour, those of Group 2 appearing the darkest, probably owing to the fact that they cohere in a compact pencil. The fact that the scale sockets are at such different levels makes the great diversity in length of the scales less apparent than is actually the case. The following measurements, however, clearly show their relative proportions. Owing to the S-shaped bend near the base of Group 2, difficulty was experienced in gauging the actual lengths of the component scales. The measurements of thicknesses were all taken at the same number of serial sections after the apex of each scale; but for purposes

of comparison, consideration must be given to the fact that while the scales of Group 2 are practically uniform, those of Groups 1 and 3 become much thicker near their bases.

Average lengths :—

Group 1	·89 mm.
Group 2	·62 mm.
Group 3	·35 mm.

Average widths :—

Group 1	·05 mm.
Group 2	·015 mm.
Group 3	·04 mm.

Average thicknesses :—

Group 1	·004 mm.
Group 2	·009 mm.
Group 3	·006 mm.

The scales of Group 1 are extremely long, fine and acuminate and the innermost are imbricated about one another in a complicated manner (fig. 26).

FIG. 25.

FIG. 25.—Scent scales from *E. elutella*, ♂. *Grp.* 1, single scale from group 1; *Grp.* 3, single scale from group 3; *Grp.* 2, entire tuft of group 2.

Those of Group 2 have blunt apices and are characterised by an S-shaped bend near their base. Some of them, however, appear to be attached directly to

their sockets without the intervention of the bend (fig. 25). As it is almost impossible to separate the scales of this tuft by mechanical means (*i.e.* by a camel-hair brush or fine needles) it is thought that their cohesion might be due to the presence of a sticky secretion on their walls. Solvents, such as potassium hydroxide, acetic acid, ether and alcohol were, however, ineffectual. The scales of the most dorsal brush are unspecialised in shape.

Microscopic structure.

All the scales have an internal chitinous meshwork, and with the exception of those in Group 2 have longitudinal striae on both faces They originate

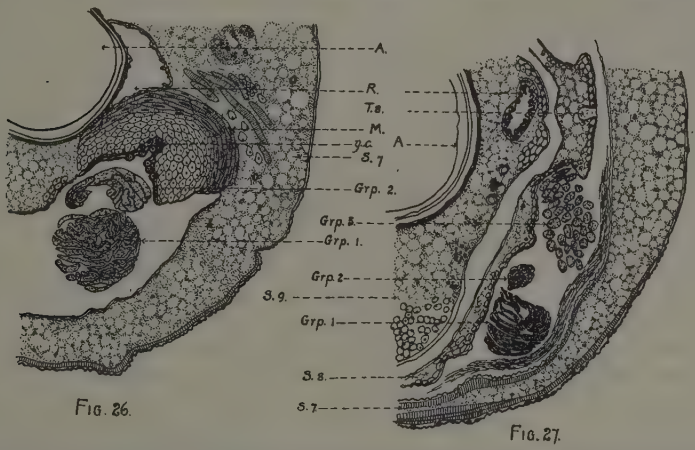


Fig. 26. Fig. 27.

Figs. 26–27.—(26) Part of a transverse section through the scent scale tufts of *E. cautella*, ♂. *A.* aedeagus; *g.c.* gland cells; *Grp.* 1, 2, scent scales of groups 1 and 2; *M.* muscle; *R.* rectum; S_7. segment seven. (27) The same as fig. 26, but more posterior. *Grp.* 3, scent scales of group 3; S_8, S_9. segments eight and nine.

in sockets embedded in a thick stratum of chitin which overlays typical gland cells. As in other species an enormous development of muscle surrounds the chitinised area to which the scales are attached (figs. 26 and 27).

Female.

The cells lining the last abdominal intersegmentum are of a glandular nature and are overlaid by clear lamellated chitin which is intermediate in thickness between the corresponding layers in *E. kühniella* and *E. cautella* (fig. 13). The surface is irregularly wrinkled and bears numbers of fine but comparatively long spines. No " glandulae odoriferae " are present.

5. Discussion.

Many of the earlier workers prefaced their descriptions of the scent apparatus in various species by an attempt to classify the different types of scent organs present. Thus Freiling (1909) and Illig (1902) grouped them according to the position in which they occurred on the body; but Urbahn (1913) separated them on difference of form and only used their position secondarily. Both

methods of classification isolate wing brands or androconia from abdominal scent organs. The presence of androconia on the wings of many Lepidoptera has been known for nearly a century, and Müller (1877) considered that in *Danaus erippus* and *D. gilippus* and probably in all other species, the development of the wing organs is in inverse ratio to that of the abdominal organs. Illig (1902) on the other hand considered the disappearance of androconia to be directly correlated with the rapidity of the wing movement.

An attempt to classify the Phycitid species used here is impracticable as only four species have been investigated. According to the degree of complexity of the scale tufts in the male, they can be arranged in an ascending series of *E. kühniella*, *P. interpunctella*, *E. elutella* and *E. cautella*. It must be stated here, however, that *E. elutella* is known to have modified scales on the hind-wings of the male. Hence it may be erroneous to consider *elutella* as more specialised than *kühniella*. If Müller's idea were correct, then one would expect *E. kühniella* and *Plodia* to have well-developed wing brands in contrast to the comparative simplicity of the tufts on the abdomen. While on the basis of Illig's supposition it would appear as though the wing movement of *E. elutella* might be less rapid than in the other species. I have no data, however, on the relative speeds of the wing movements.

As I have not yet investigated the modified wing scales of *E. elutella*, the information I have given is concerned solely with abdominal scent organs. These are manifest in the males, as has been described, in the form of tufts of modified scales subtended by glandular hypoderm cells. In the females there are no specialised tufts, and indeed, as Urbahn (1913) stated, it is rarely they occur in female Lepidoptera. It is noteworthy, however, that Freiling (1909) described scent brushes in the female of *Gonepteryx rhamni* in the intersegmental fold between the seventh and eighth abdominal segments, close to the entrance to the bursa copulatrix. In addition, Richards and Thompson (1932) describe a similar brush in the female of *E. figulilella* as " a large firmly attached fan of modified scales, arising on each side of the ostium from a narrow chitinised strip in the membrane between the seventh and eighth sternites." Unfortunately I have not seen a specimen of *E. figulilella*, but apparently there are no scent scale tufts on the abdomen of the male.

In describing the structure of the scent apparatus I have made no mention of the method of diffusion of the scent. Reichenau (1880), working on the male of *Sphinx ligustri*, supposed the scent material to be pressed by muscular action from the gland into the hairs or scales and thence diffused to the atmosphere through their apices. He also maintained that the scales were provided with capillary tubes in which the peculiar scent substance was held in the form of bubbles. These bubbles of Reichenau's were doubtless the chitinous reticulum which is so evident in the scales of *E. cautella*, and which probably holds the secretion within its meshes. This tacit assumption that the scent scales are provided with apical pores was authenticated by Freiling (1909), who figured a terminal pore in the scent scales of *Notris verbascella*. Likewise he described numerous pores situated between the striae of the scent scales of *Gonepteryx rhamni* (female) similar to those that Eltringham (1915) found in the modified hairs of *Tirumala petiverana*. Even before these modified scales were identified as scent-disseminating, pores had been described in them. Thus Illig (1902) quotes the work of Watson (1872), who attributed to the pores in the modified scales of certain exotic Lepidoptera the function of pumping air into the tracheae.

Even under the highest magnification I can find no pores in the scales of

E. kühniella, *E. elutella* or *P. interpunctella*. With *E. cautella*, however, I am inclined to believe that small openings do occur between the longitudinal striations on the scales of Groups 4 and 5. After cutting sections of the scales, it appeared that these "pores," seen externally as tiny pinpricks of light, could be identified with a single horizontal bar of the internal chitinous reticulum. Since examining them with an oil immersion lens I now consider my original opinion (that they were actual pores) to be correct. I can give no reason why pores should be present in some of the scales in *E. cautella* and absent in the scales of the other species. Hence I must presume either that they occur on all scales but are too small to be seen, or that they are absent in all scales, and their supposed occurrence in *E. cautella* is fallacious. In the latter case, one is forced to uphold the theory that the scent substance diffuses through the whole chitinous surface of the scale. This is in accord with the idea so often stated that the larger size of the scent scale (as compared with that of the clothing scale) provides an increased area for the evaporation of the scent material.

In *Lycorea atergati* Illig (1902) described a small passage between the stalk of each scent scale and its alveolus; *i.e.* the socket is larger than the contained pedicel. He assumed that the scent material could diffuse from the gland through this passage, and that the external ribs of the scale served as an enlarged catchment area for the secretion. There seems to me to be very little foundation for such a theory, and it entirely ignores the fact that the scale pedicel is hollow. Illig himself, in the male of *Euploea*, acknowledged the presence of a lumen, which he described as being comparatively wide near the base of the scale but became much narrower near the apex. Despite such differences in opinion on the actual method of dissemination all workers agree with Müller's statement (1877) that "one could hardly find a more effective method of employing any odoriferous substance, than that of saturating with it the hairs of a brush, and then suddenly opening them out in all directions, so as to provide an enormous surface for evaporation."

With regard to the dissemination of the scent from the membranes of the female—the thick chitinous stratum, which in all four species lines the intersegmental fold between the eighth and ninth abdominal segments, appears to hermetically seal the underlying glandular epithelium from the exterior. Hence the presence of pores in it would seem to be essential. The corresponding layer in the wax glands of aphids and bees is studded with pores each of which is the opening of a single gland cell, and by means of a freezing microtome Wigglesworth has demonstrated their presence in *Rhodnius* sp., etc. (1934). Yet there are no records of homologous structures having been found in the scent glands of female Lepidoptera. Urbahn (1913) used various illuminating methods including dark ground illumination with an Ultra microscope, but failed to discover pores. He therefore concludes that the scent material soaks through the lamellae of the chitinous layer until it reaches the epiderm, through which it slowly diffuses. Eltringham holds the same views. This theory seems to me adequately to account for the fact that in many instances I have noted that this stratum will absorb a stain when other chitinous structures remain unstained. It would thus appear that the chitin is impregnated with a secretion which is responsible for the absorption of the stain. The thicker the chitinous layer the more secretion it is capable of holding; hence it is assumed that the degree of development of this layer is proportional to the activity of the gland cells. If the so-called "glandulae odoriferae" are also concerned in the production of a volatile secretion

attractive to the male, then the combined output of these glands and the intersegmental fold in *E. cautella* must be much greater than in the other species.

It is extraordinary that in the male the scent apparatus is usually so much more specialised than in the female; and it is only very rarely that both sexes possess the organs in a similar state of development, such as Philpott (1924) describes in *Sabatinca*, *Epimartyria*, *Mnemonica* and *Eriocrania*. It has been doubted whether the intersegmental folds of the distal abdominal segments of the female could alone be responsible for the scent which is apparently needed to attract the males and hence ensure the continuance of the species. It is known that with diurnal species of Lepidoptera sight must play a part, and there has even been a suggestion that sound is used in sexual attraction. Stobbe (1912) quotes many early scientists who believed the abdominal scent organs of the male to be a vocal apparatus, and this idea was prevalent until Müller (1877) proved the hair tufts of a Brazilian Zygaenid to be scent-producing. That this theory is not without some foundation has been confirmed by Petersen (1904), who stated that he heard noises issuing from certain tropical Nymphalids when the sexes were seeking one another. He considered the development of the large tympanum common to many Noctuids, Geometrids, Phycitids, etc., to be in accordance with this phenomenon.

It was early surmised (Siebold, 1837), and has since been frequently proved, that, with the exception of *Hepialus hectus* and possibly *Biston hittarius* (Richards, 1927), the odour emitted by the female is to entice the male, while that emitted by the male is used solely as a stimulus in copulation. Urbahn (1913), however, considered it probable that scent glands in some instances might serve the double function of sexual attraction and a repulsive purpose. Dixey (1910) thought this to be unlikely as he has noted that (with few possible exceptions) the scents that are believed to be attractive to the opposite sex are agreeable to the average human perception. Eltringham (1926) refuted this idea of Dixey's, but supports Urbahn's view, when he says that "the fact that the fresh secretion of the glands in *Heliconius* is unpleasant to the human sense, is not necessarily evidence that it may not have an attractive or an excitative function in the economy of the insects themselves. Such a product might serve the double purpose of an amatory or repugnatorial function."

These hypotheses are beyond the scope of the present paper, as apart from all other considerations the scent emitted by *Ephestia* and *Plodia* is imperceptible to the human sense. Nevertheless it is interesting to note in this connection that several observers (Dixey, Longstaff, etc.) consider the scents of many Lepidoptera to resemble those of certain plants. Petersen (1904) even went so far as to correlate the odour of the adult with that of its food-plant in the larval stage. This, of course, if correct, can only apply to strictly monophagous species, and the wide variety of food-stuffs consumed by *Ephestia* and *Plodia* could have no influence on the adult odour.

It is a source of wonder that two such closely allied species as *E. kühniella* and *E. cautella* could have such a different degree of specialisation of the scent apparatus. It has been pointed out by Petersen (1904) that the large variety of scent organs is not so astounding when one considers the enormous variety of scent materials which are produced. It is obvious that in order to maintain its specific status, a species must be able to recognise, and be attracted, only by the scent from a female of its own species. In other words, the scent emitted by a female must be specific. Yet there are cases on record which appear to be contrary to this statement. Withycombe (1926) states that he

had seen male ZYGAENIDAE attracted by a female of *Lasiocampa quercus*, and that Carpenter had found a male of the African *Acraea terpsichore* attempting to pair with a male Syntomid—*Epitoxis albicincta*. Petersen (1904) described some very interesting experiments carried out by Standfuss in 1896 on this phenomenon. He exposed two freshly emerged females of different species—*Callimorpha persona* and *C. dominula*—and then released several *persona* males in their vicinity. He found that they were equally attracted to both females. Similarly he had seen two not closely related species, such as *Sphinx ligustri* and *Smerinthis ocellata*, attracted to one another and even effecting copulation. Further, Standfuss discovered that the cross between *Saturnia pyri* and *S. pavonia* (*Saturnia* hybrid *emiliae*) possessed a physiological affinity to the opposite sex of both the parent species so that they become reabsorbed into the original stock. He considered these observations substantiated his view that the scent in the local races of one species had a tendency to diverge from the stock type. Austin Clark (1926) thought it quite possible that in certain species there will be found to be a geographical variation in the strength or type of the odour, which may or may not be correlated with variation in colour or in other characteristics. This may be one of the factors concerned in the origin of a new species.

6. EXPERIMENTAL WORK.

Experiment A.

The aim was to determine whether the male of one species could be attracted by the female of another.

Method.

(i) Into a glass-topped tin container (well sterilised before using) were put :—

(*a*) A female of *E. elutella*, a virgin, aged 48 hours and in the " calling " condition.

(*b*) A female of *E. cautella*, virgin, aged 48 hours and in the calling condition.

(*c*) A female of *E. kühniella*, virgin, aged 24 hours, not calling.

A male of *Plodia interpunctella* (aged 48 hours) was introduced and immediately showed all the symptoms of excitement (running in all directions, fluttering wings and vibrating antennae) which are usually manifest in response to the odour emitted by a calling female of its own species. It disturbed the three females, causing *elutella* and *cautella* to stop calling for a time. Repeated efforts were made to pair with the *kühniella* female, stroking her antennae and pushing her abdomen with his head. Attempts were also made to copulate with the female *cautella*, which was unresponsive and the male returned again and again to *kühniella*. These efforts continued for two hours fifteen minutes.

This experiment was repeated with the following combinations of individuals in the one container :—

(ii) (*a*) female *cautella*, 36 hours, not calling.
(*b*) female *cautella*, 30 hours, calling.
(*c*) female *Plodia*, 52 hours, calling.
(*d*) female *elutella*, 48 hours, calling.
(*e*) female *elutella*, 12 hours, not calling.

(All virgin except (*d*) which had previously paired.)

Male *kühniella*, 36 hours old, introduced.

(iii) (*a*) female *kühniella*, 42 hours, calling
(*b*) female *elutella*, 48 hours, not calling.
(*c*) female *cautella*, 24 hours, calling.
Male *Plodia*, 24–27 hours, introduced.

(iv) (*a*) female *Plodia*, 36 hours, calling.
(*b*) female *kühniella*, 52 hours, calling.
(*c*) female *elutella*, 52 hours, not calling.
Male *cautella*, 24 hours, introduced.

In all the above instances the male became excited without the presence of a female of its own species to stimulate it, but although repeated attempts were made to pair, none was successful. This was no doubt due to physical incompatibility.

The conclusion is that a male of one species can be attracted by a female of another species.

Experiment B.

The aim was to determine whether a male of one species can differentiate between the scents emitted by a female of its own species, and that emitted by females of closely allied species.

Method.

The same specimens as had been used in expt. A. When the male *Plodia* was removed, a male *cautella* (3 days old) was introduced into the container. The removal of the one and the substitution of the other male disturbed the females from the calling position. Twenty minutes elapsed before the male suddenly became excited and ran to the *kühniella* female with fluttering wings and vibrating antennae. He bent his abdomen across his back and tried in vain to copulate. Three minutes later the female *cautella* recommenced calling and attracted the male and they were in copulation within one minute. Apparently he was stimulated to excitement in the first place, either by the scent of the female *cautella*, or by the accumulation of the scents of all the females within the tin, but that on the female *cautella* recommencing to call his attention was immediately engaged.

The conclusion is that a male of one species can differentiate between the scents emitted by a female of its own species and that emitted by females of closely allied species.

Experiment C.

The aim was to determine whether a male is attracted by the sight of a female, or whether he merely perceives some odour which might emanate from her.

Method.

(i) A calling female *cautella* (virgin, 2 days old) was placed in a glass-topped tin together with a dead male *cautella* and several empty pupal cases. Fifteen minutes later a male *cautella* (2 days old) was introduced and he immediately became excited and began to seek the female. The scent she was emitting was apparently the stimulation, but he could not at first discover its source. Thus he made repeated attempts to pair with the dead specimen and the pupal cases, before locating the female.

(ii) A female *kühniella* (virgin, 2 days old) which had been calling, was wrapped in loose cotton wool and put in a freshly sterilised box. Twenty

minutes later a male *kühniella* (3 days old) was introduced. Although the female was quite invisible he fluttered round the cotton wool for 25 minutes before he became passive.

(iii) The eyes of two male *kühniella* (aged 48 and 52 hours respectively) were painted with a double coat of Indian ink. Both specimens were then put into a box containing a virgin 24-hours-old *kühniella* female, which had been calling but ceased on the entrance of the males. They both became excited and after two and a half hours of alternate periods of excitement and passivity, one of them effected copulation.

The conclusion is that a male is attracted by the odour and not by the sight of a female.

Experiment D.

The aim was to determine whether the scent which attracts the male emanates from the abdomen of a female.

Method.

The abdomens of two virgin *kühniella* females (aged 4 and 2 days respectively) were put at one end of a glass-topped box. The head and thorax, with wings attached, of both specimens were placed at the other end. Both ovipositors were vibrating in a manner similar to that observed when a female is in the calling condition. A male (36 hours) was introduced and he immediately became excited and fluttered over the abdomens, entirely ignoring the anterior halves of the females. Many attempts were made to copulate, but these failed owing to the fact that normally a male commences by approaching the female head on, grasping her by the thorax, and then bending his abdomen across her back. The absence of the head and thorax of the female frustrated these manœuvres.

This experiment was repeated about a dozen times and only twice was pairing effected. On one occasion the pair remained in copulation for sixteen minutes only, and on the other they did not separate before death intervened. Dissection of both abdomens showed that no spermatophore had been introduced into the bursa copulatrix. It is interesting to note that one abdomen of a four-day-old *kühniella* female lived for eight days after it had been severed. The ovipositor ceased to vibrate after the fourth day.

The conclusion is that the scent which attracts the male emanates from the abdomen of the female.

Experiment E.

The aim was to determine what effect the absence of the scent tufts in the male has upon copulation.

Method.

By slightly anaesthetising the following adult male Phycitids the terminal segments were pulled out from their invaginated position and the scent tufts removed by fine scissor forceps—three specimens of *E. kühniella*, two of *E. elutella*, one of *E. cautella* and four of *P. interpunctella*. As a control six specimens (four *kühniella* and two *Plodia*) were anaesthetised but were left unmutilated. All specimens were left for twenty-four hours to recover from the effects of the anaesthetic. At the end of this period two of the controls (one of each species) and one *kühniella*, one *cautella*, and two *Plodia* were dead. The remainder were then placed in a box containing several females of the different species. Two of the *kühniella* specimens used as controls effected copulation.

One *Plodia* and one *elutella* of the mutilated specimens showed all symptoms of excitement. The *Plodia* made no attempt to pair, but the *elutella* male did—the female concerned, however, remained quite unresponsive. The evidence of this experiment is liable to misinterpretation. The very small size of the specimens made removal of the scent tufts without inflicting other injuries extremely difficult. If, however, the female *elutella* remained impassive to the importunities of the male solely because the necessary stimulus was lacking, I conclude that :—

(i) the modified tufts of the male emit a scent which acts as a stimulus to copulation; and (ii) the absence of the scent tufts in the male prevents copulation.

Experiment F.

The aim was to determine whether a female increases in attractiveness as she becomes older.

Method.

Six glass-topped tins all of the same volume, viz. 122·53 c.c., were thoroughly sterilised. A virgin female was put into each tin at 10.30 a.m. Their respective ages were :—

Tin (*a*) female *kühniella*—freshly emerged.
Tin (*b*) female *kühniella*—22 hours old.
Tin (*c*) female *kühniella*—36 hours old.
Tin (*d*) female *kühniella*—42 hours old.
Tin (*e*) female *kühniella*—58 hours old.
Tin (*f*) female *kühniella*—over 4 days old.

Six males, emerged between 9–11 p.m. the night before, were introduced one into each tin. With the exception of females in (*a*) and (*e*) all were calling. The entrance of the male into tin (*e*) induced the female to call, and although such a phenomenon is rare it has been noted on other occasions.

Before 11.5 a.m. the pairs in tins (*e*) and (*f*) were in copulation. By 11.46 a.m. the pair in tin (*c*) were in coitu, and the two in tin (*a*) commenced pairing some time between 7.30 and 9 p.m. Next morning the females in tins (*b*) and (*d*) were dissected, and a spermatophore was found in the bursa of (*b*)—it was concluded that copulation had taken place during the night. For some unknown reason the female in (*d*) had failed to pair.

The conclusion is that a female over 50 hours old is more attractive than one freshly emerged.

Experiment G.

The aim was to determine whether a newly emerged male reacts as quickly as an old male to the presence of a female.

Method.

At 11.10 a.m. four *kühniella* females each aged 24 hours were put into containers of the same volume (122·53 c.c.). At 11.30 a.m. two newly emerged males and one three days old and another four days old were introduced one into each tin. Within sixteen minutes of his entrance the three-day-old male had effected copulation. Ten minutes later the four-day-old male had commenced to pair. Both the freshly emerged males paired some time during the following night.

The conclusion is that the time taken for an old male to react to the presence of a female is less than that taken by a freshly emerged male.

Experiment H.

The aim was to determine whether the length of time that a female has been in a container prior to the entrance of a male, affects his reaction.

Method.

The results of this experiment are based on evidence gathered from many experiments in which females of the same age were placed in the same sized containers for varying lengths of time prior to the introduction of a male of a standard age.

Observations.

This determination is affected by several factors. In the first place the size of the container is important. As nothing is known about the density of the scent emitted by the female we do not know whether it is rapidly diffused throughout the container, or whether it has a tendency to settle in a layer about the female. If it has the latter tendency, it is probable that the air immediately surrounding the female will become supersaturated with the volatile substance, which may then act as a check to the rapidity of further dissemination. In which case the distance between the introduced male and the female, and not the size of the container, is the important factor. If, on the other hand, the scent diffuses rapidly throughout the container, a male introduced a short time after another male will probably not react any faster than the first. Finally, comparatively long intervals of time between the introduction of respective males brings in the factor of the age of the female (*see* expt. F). Despite these considerations I must conclude from the evidence that :—

A male will react more rapidly to a female which has been calling for some time in the box prior to his entrance, than to a female added to a box at the same time, or slightly before, the addition of the male.

This must be due to the accumulation of the scent of the female within the box.

7. Remarks on Experiments.

The preceding experiments are not based on any single set of observations, as it was found that several unexpected factors were involved. Thus occasionally both males and females were obtained that made no attempt to pair throughout their lives, due either to mechanical or physiological defects. The work of M. J. Norris (1933) shows that there is a possible correlation between the quantity of sperm present and the readiness of a male to pair. Similarly " calling " appears to be dependent on the shortage of sperm in the receptaculum seminis.

It is evident that the scent of the females is only emitted when they are in the calling position, this fact having been noted on many different occasions by various observers (Haase, 1880; Dalla-Torre, 1885; Freiling, 1909; Norris, 1933). Correspondingly the scent of the males of *P. interpunctella, E. kühniella* and *E. elutella* must be emitted when the terminal segments are exserted and the claspers opened wide ready to grasp the female. It has been noticed repeatedly that in the male of *E. cautella* is manifest an additional symptom of excitement. Thus, besides vibrating the antennae and fluttering the wings, it bends the abdomen in a dorsal direction similar to the calling position of the female. This movement exposes the scent scales and presumably scent is emitted.

It is probable that calling and pairing are not solely dependent on the quantity of sperm, age of male or female, or on a chemotropic stimulus, but that the conditions under which the insects were reared, as determined by temperature, humidity, and light intensity, are all involved. Thus, if a culture of *Ephestia* was reared under crowded conditions the attendant rise in temperature resulted in a large percentage of individuals refusing to pair. Whether this is due to

(1) morphological defects in the genitalia,
(2) physiological defects, or
(3) absence or non-functioning of the scent glands in the female,

has been discussed by Norris (1933). Insects reared under normal conditions but at high temperatures (27°) were found by Raichoudhury (1933) to copulate readily, but such pairing was not followed by the laying of viable eggs. He established that this infertility at high temperatures in *Ephestia* was associated with a retardation of spermatogenesis in the male.

The effect of humidity on sexual activity has been studied by Holdaway (1932), who observed that if the relative humidity is low at the normal time for pairing, mating did not take place. If, however, the relative humidity rose during the interval between normal mating and oviposition, sexual activity was immediately aroused.

That the phototropic stimulus affecting copulation is negative, was noticed repeatedly during the course of these experiments. Strong light intensity hindered both calling and readiness to pair. Hence, experimental work was conducted in the constant temperature room where standard conditions of humidity, temperature and light intensity were maintained.

8. Summary and Conclusions.

1. The scent apparatus in the male of *Ephestia kühniella*, *E. elutella*, *E. cautella* and *Plodia interpunctella* is in the form of tufts of modified scales arising from small chitinised areas one on either side of the anterior prolongation of the eighth tergite.

2. The scent apparatus of the females of the same species is in the form of a glandular intersegmental fold between the eighth and ninth segments. In addition the female of *E. cautella* has two internal odoriferous glands in the last abdominal segment.

3. Dissemination of the scent takes place in both male and female by diffusion through the cuticle of the structures concerned.

4. Dissemination of the scent occurs in the female when she is in the "calling" position and the membranes between the apical segments of the abdomen are fully exposed.

5. Dissemination of the scent in the male occurs when the genitalia are opened in readiness to pair. In *E. cautella* the scent scales are exposed when the male first reacts to the presence of the female.

6. A male of one species can be attracted by a female of another species.

7. A male of one species can differentiate between the scents emitted by a female of his own species and that emitted by females of closely allied species.

8. A male is attracted by the odour and not by the sight of a female.

9. The scent which attracts the male emanates from the abdomen of the female.

10. The modified tufts of the male emit a scent which acts as a stimulus to copulation.

11. The absence of the scent tufts in the male prevents copulation.

12. A female increases in attractiveness as she becomes older.

13. The time taken for an old male to react to the presence of a female is less than that taken by a freshly emerged male.

14. A male will react more rapidly to a female which has been "calling" for some time in the box prior to his entrance, than to a female added to the box at the same time, or slightly before, the addition of the male. This must be due to the accumulation of the scent of the female within the box.

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